Standard Operating Procedure for the Analysis of PCB Congeners by GC/ECD and Trans-Nonachlor by GC/MS/ECNI

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1.0 Scope and Application

1.1 Scope

This method is used to determine the concentrations of PCB congeners and trans-nonachlor in extracts from phytoplankton, zooplankton, *Mysis*, *Diporeia*, detritus and dissolved phase of lake water samples. As the detritus and dissolved phase parameters are not funded by this project, procedures relating to these parameters are provided as information only. The following analytes are measured by this Standard Operating Procedure (SOP):

Analyte	**CAS #**
trans-nonachlor	39765-80-5
PCB Congener #	**CAS #**
1	2051-60-7
3	2051-62-9
4	13029-08-8
5	16605-91-7
6	25569-80-6
7	33284-50-3
8	34883-43-7
9	34883-39-1
10	33146-45-1
12	2974-92-7
13	2974-90-5
14	34883-41-5
15	2050-68-2
16	38444-78-9
17	37680-66-3
18	37680-65-2
19	38444-73-4
21	55702-46-0
22	38444-85-8
24	55702-45-9

PCB Congener #	CAS #
25	55712-37-3
26	38444-81-4
27	38444-76-7
28	7012-37-5
29	15862-07-4
30	35693-92-6
31	16606-02-3
32	38444-77-8
33	38444-86-9
37	38444-90-5
40	8444-93-8
41	52663-59-9
42	36559-22-5
43	70362-46-8
44	41464-39-5
45	70362-45-7
46	41464-47-5
47	2437-79-8
48	70362-47-9
49	41464-40-8
51	68194-04-7
52	35693-99-3
53	41464-41-9
56	41464-43-1
60	33025-41-1
63	74472-34-7
64	52663-58-8
65	33284-54-7
66	32598-10-0
70	32598-11-1
71	41464-46-4
74	32690-93-0
76	70362-48-0
77	32598-13-3
81	70362-50-4

PCB Congener #	CAS #
82	52663-62-4
83	60145-20-2
84	52663-60-2
85	65510-45-4
87	38380-02-8
89	73575-57-2
91	68194-05-8
92	52663-61-3
95	38379-99-6
97	41464-51-1
99	38380-01-7
100	39485-83-1
101	37680-73-2
105	32598-14-4
107	70424-68-9
110	38380-03-9
114	74472-37-0
118	31508-00-6
119	56558-17-9
123	65510-44-3
124	70424-70-3
128	38380-07-3
129	55215-18-4
130	52663-66-8
131	61798-70-7
132	38380-05-1
134	52704-70-8
135	52744-13-5
136	38411-22-2
137	35694-06-5
138	35065-28-2
141	52712-04-6
144	68194-14-9
146	51908-16-8
147	68194-13-8

PCB Congener #	CAS #
149	38380-04-0
151	52663-63-5
153	35065-27-1
156	38380-08-4
157	69782-90-7
158	74472-42-7
163	74472-44-9
166	41411-63-6
167	52663-72-6
170	35065-30-6
171	52663-71-5
172	52663-74-8
173	68194-16-1
174	38411-25-5
175	40186-70-7
176	52663-65-7
177	52663-70-4
178	52663-67-9
180	35065-29-3
182	60145-23-5
183	52663-69-1
185	52712-05-7
187	52663-68-0
189	39635-61-9
190	41411-64-7
191	74472-50-7
193	69782-91-8
194	35694-08-7
195	52663-78-2
196	42740-50-1
197	33091-17-7
198	68194-17-2
199	52663-75-9
200	52663-73-7
201	40186-71-8

PCB Congener #	CAS #
202	2136-99-4
203	52663-76-0
204	74472-52-9
205	4472-53-0
206	40186-72-9
207	52663-79-3
208	52663-77-1
209	2051-24-3

1.2 Method Optimization

The analyst selects columns and calibration procedures most appropriate for the specific analytes of interest in a study. Matrix-specific performance data are established and the stability of the analytical system and instrument calibration are established for each new matrix.

1.3 Resolution

The analytes listed in Section 1.1 are detected in a clean matrix. Some analytes co-elute with other analytes so that one peak is identified as more than one PCB congener. Analytes that can be detected and quantified in individual samples may differ. This is due to the possible chemical and chromatographic behavior of many of these analytes in real environmental matrices. Cleanup/fractionation schemes are provided in this method.

1.4 Compound Identification

Identification based on single column GC analysis should be supported by one other qualitative technique. Qualitative support for PCB identification will be provided by GC/MS/ECNI. Confirmation of selected PCB congeners with more than four chlorines will be conducted on 5% of the samples. The GC/MS/ECNI method for the analysis of trans-nonachlor directly confirms the identity of the compound, so external confirmation is not needed.

1.5 Method Usage

This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph (GC), mass spectrometer (MS) and in the interpretation of gas chromatograms and mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.6 Working Linear Range

It is important to ensure linearity between a range of PCB concentrations. To demonstrate linearity, five calibration standard solutions ranging from approximately 300 ng/mL to 3000 ng/mL will be run on the GC and compared on a congener basis.

1.7 Limit of Detection Terminology

This study will incorporate three terms describing detection limits - Instrument Detection Limit (IDL), Method Detection Limit (MDL) and System Detection Limit (SDL) - see Section 13.2 for specific calculations. Definitions for each term follows:

IDL = y-intercept of initial calibration curve

MDL = three standard deviations of seven injections of low level homolog spikes, adjusted for congener specific relative response factors (RRF)

SDL = three standard deviations of seven injections of field matrix blanks

2.0 Summary of Method

All samples will undergo methanol rinse, four hour Soxhlet extraction with methanol and 16 to 24 hour Soxhlet extraction with dichloromethane (DCM). Surrogate compounds (congeners 14, 65, 166 for PCBs and ^{13}C-chlordane or ^{37}Cl-nonachlor for trans-nonachlor) are added at the beginning of the extraction to monitor the efficiency of the extraction process. The methanol fraction is batch extracted with hexane. Water/methanol phase is discarded. The hexane fraction containing PCBs is saved, combined with the DCM fraction, solvent exchanged to hexane and volume reduced to approximately 15 mL.

Extracts have lipids removed by passing them over a column containing:

1g Na_2SO_4
13g 6% deactivated Al
1g Na_2SO_4
Washed with 2 x 60 mL hexane

The columns are eluted with 150 mL hexane, and the extracts are volume reduced to approximately 10 mL.
The extracts undergo column chromatography to separate PCBs from nonachlor and toxaphene and to aid in further cleanup. The extracts are loaded onto the following column:

3g Na_2SO_4
4.5g 0% deactivated Si
1g Na_2SO_4
6g 1% deactivated Al
1g Na_2SO_4
Washed with 2 x 50 mL 2% DCM/hexane, 2 x 50 mL 40% DCM/hexane, 2 x 60 mL 100% hexane.

The columns are then eluted with 95 mL 100% hexane followed by 105 mL 40% DCM/hexane.

Both fractions are solvent exchanged and volume reduced to approximately 1 mL. The final extracts are stored in amber vials in the freezer until analysis. Just prior to instrumental analysis, extracts are reduced to approximately 200-300 µL by a gentle stream of nitrogen and internal standards are added. Internal standards used are PCB congeners 30 and 204 for PCBs and 204 for trans-nonachlor.

PCBs will be quantified by individual congener using the method of Mullin (1985). The 1994 congener standard supplied by Mullin will be used as the quantitation, performance, and spiking standard. Analysis will be accomplished using a Hewlett Packard GC equipped with an ^{63}Ni electron capture detector (ECD) and a 60 m high resolution capillary column (DB-5) with H_2 carrier gas. Individual congener data are processed by Millennium Chromatographic Management System (Waters Corporation). Concentrations will be reported by individual congener, homologe distributions and total PCBs.

Trans-nonachlor will be analyzed by electron capture negative ionization (ECNI) GC/MS with selected ion monitoring (SIM). The GC utilizes a 60m DB-5 MS column with He carrier gas. Methane is the reagent gas. Further instrument specifics can be located in Section 5.19. Mass spectra are acquired and processed by Hewlett Packard (HP) RTE-A and Aquarius software.

3.0 Interferences and Corrective Action

3.1 Sources of Interference

Sources in this method can be grouped into three broad categories: contaminated solvents, reagents, XAD resin, or sample processing hardware; contaminated GC carrier gas, parts, column surfaces or detector surfaces; and the presence of coeluting compounds in the sample matrix to which the ECD will respond. Interferences coextracted from the samples will vary considerably from sample to sample. While general cleanup techniques are provided as part of this method, unique samples may require additional cleanup approaches to achieve desired degrees of discrimination and quantitation.

3.2 Interferences by Phthalate Esters

These interferences which are introduced during sample preparation can pose a major problem in PCB determinations. These materials may be removed prior to analysis using the silica gel/alumina cleanup. Common flexible plastics contain varying amounts of phthalate esters which are easily extracted or leached from materials during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalate esters can best be minimized by avoiding contact with any plastic materials. Exhaustive cleanup of solvents, reagents and glassware may be required to eliminate background phthalate ester contamination.

3.3 Glassware

Glassware must be scrupulously cleaned. Used glassware is cleaned with Alconox detergent in hot water, rinsed with tap water followed by deionized water. The glassware is allowed to dry, is foil wrapped, and ashed for a minimum of four hours at 450°C. It is then stored in a clean environment.

3.4 Toxaphene

The presence of toxaphene will result in a series of peaks that interfere with the detection of PCBs. Toxaphene and PCBs will be separated by column chromatography using a silica gel/alumina column. PCBs will be recovered in the first elution fraction while toxaphene will elute in the second fraction.

4.0 Safety Precautions

4.1 Safety Attire

Latex disposable gloves and lab coats are worn when making up stock solutions of standards. Lab coats are preferred but optional during other activities. Gloves will not be worn during other steps in the method due to the possibility of sample contamination resulting from materials in the gloves.

4.2 Equipment Testing

Testing of the ^{63}Ni ECD is performed bi-annually by the University of Minnesota Radiation Protection Division. Testing of the hoods is performed by the University of Minnesota Environmental Health and Safety Division on a bi-annual basis.

5.0 Apparatus and Materials

5.1 Soxhlet Extractor

Soxhlet extractors used are 50 mm ID with 500 mL round bottom flask. This will be used with all matrices except for XAD which will use 68 mm ID with 1000 mL round bottom flasks. A plug of ashed glass wool is placed in the bottom of the Soxhlet before the sample is introduced.

5.2 Kuderna-Danish (KD) Concentrator

The KD apparatus consists of three parts as described below.

5.2.1 Receiver

Either 10 mL or 15 mL. Ground glass stoppers of the appropriate size are used to prevent evaporation of extracts after volume reduction.

5.2.2 KD Body

Either 500 mL or 250 mL. The evaporation flask is attached to the receiver with delrin clamps.

5.2.3 Snyder column

Three ball macro. Ground glass stoppers of the appropriate size are used to prevent contamination if volume reduction must be interrupted before completion.

5.3 Lipid Removal

An initial cleanup column (1 x 50 cm) is used to remove lipids comprised of 6% deactivated alumina and anhydrous sodium sulfate.

5.3.1 Sodium sulfate

Anhydrous granular, Mallinckrodt, pre-ashed at 450°C for four hours.

5.3.2 Alumina

60-325 mesh, Fisher Scientific, pre-ashed at 450°C for four hours.

5.4 Cleanup Columns

Cleanup columns - 1 cm x 50 cm. Columns are comprised of a small plug of ashed glass wool, ashed anhydrous sodium sulfate, 1% deactivated alumina and 0% deactivated silica gel.

5.4.1 Sodium sulfate

Anhydrous granular, Mallinckrodt, pre-ashed at 450°C for four hours.

5.4.2 Alumina

60-325 mesh, Fisher Scientific, pre-ashed at 450°C for four hours.

5.4.3 Silica gel

60-200 mesh, Baker Analyzed, pre-ashed at 300°C for four hours.

5.5 Nitrogen Evaporation Apparatus

Pierce Reacti Therm Model 18780 Evaporating Unit. Ultra-pure Carrier grade nitrogen is used at a flow setting of 7 psi.

5.6 Boiling Chips

Eight to 12 mesh, Cargille Laboratory, ashed at 450°C for a minimum of four hours.

5.7 Heating Mantle

Electrothermal Soxhlet Apparatus heater. Individual solid-state controllers control the heat for each of six individual heating bays.

5.8 Steam Bath

Heated, concentric ring cover. Steam bath is used in the hood.

5.9 Extract Vials

Extracts are stored in 4 mL amber vials which have been wrapped in aluminum foil and ashed at 450°C for a minimum of four hours.

5.10 Drummond Pipets

Drummond pipettes are used to deliver surrogates and internal standards in amounts of 10 µL, 25 µL, 50 µL, and 100 µL.

5.11 Vortex Mixer

Deluxe Mixer by Scientific Products.

5.12 Apparatus for Determining Percent Dry Weight:

5.12.1 Sartorius MC1 Balance

Self calibrating.

5.12.2 Drying oven

Precision Scientific, drying is performed at 60°C.

5.13 Apparatus for Determining Percent Lipid:

5.13.1 Sartorius MC1 Balance

Self calibrating.

5.13.2 Drying oven

Precision Scientific, drying is performed at 60°C.

5.13.3 Aluminum weighing tins

Fisherbrand.

5.14 SPM Determination:

5.14.1 0.4 µm Nuclepore filters, 47 mm diameter

5.14.2 Sartorius MC1 Balance

5.15 POC Apparatus

Leko Total Carbon Analyzer

5.16 DOC Analyzer

Ionics Model 555, Thermal Combustion, Total Carbon Analyzer.

5.17 Gas Chromatograph

Following are the gas chromatograph parameters.

Model:	HP 5890A
Injector:	Splitless
Injector Temp.:	225°C
Detector Temp.:	325°C

Temperature Program:	100°C hold for 10 minutes
	100°C-130°C at 10°/min.
	130°C-255°C at 1°/min.
	255°C-285°C at 10°/min.
	160 minutes for a run
Injection Volume:	1 µL
Carrier Gas:	H_2
Processing System:	Millennium 2010 Chromatography Manager

5.18 Narrow-bore columns:

5.18.1 GC Column 1

60 m x 0.25 mm internal diameter (ID) fused silica capillary column DB-5 (J & W Scientific) chemically bonded with 5%-Phenyl Methylpolysiloxane, 0.25 µm film thickness.

5.18.2 GC/MS/ECNI Column

60m x 0.25 mm ID fused silica capillary column DB-5 MS (J & W Scientific), 0.25 µm film thickness.

5.19 GC/MS/ECNI

Following are the parameters for the MS system.

Model:	Hewlett Packard 5988A
Autosampler:	HP 7637
Mode:	Single Ion Monitoring (SIM), Negative Ion Mode
Injector:	Splitless
Injector Temp.:	270°C
Temperature Program:	80°C hold for one minute
	80°C-210°C at 10°/min.
	210°C-250°C at 0.8°/min.
	250°C-290°C at 10°/min.
Injection Volume:	1 µL
GC Carrier Gas:	He
NI Reagent Gas:	Methane
Transfer Line Temp:	290°C
Source Temp:	100°C
Source Pressure:	1.2 Torr
Electron Energy:	240 EV
Emission Current:	300 A
Mass Range:	1-800 m/z
Mass Accuracy:	0.3 ± dalton
Scan Start Time:	24 minutes

6.0 Reagents

6.1 Chemicals

Reagent or pesticide grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

Note: Standard solutions (stock, calibration, internal, and surrogate) are stored at -16 ±4°C in amber glass containers. When stock standard solutions are prepared, it is recommended that aliquots of that stock solution be stored in individual small vials for use as working solutions. Standard solution must be replaced if routine QC indicates a problem.

6.2 Solvents

All solvents should be pesticide quality or equivalent. Solvents must be exchanged to hexane prior to analysis.

6.2.1 Hexane

Fisher Optima.

6.2.2 DCM

Fisher Optima.

6.2.3 Methanol

Fisher Optima.

6.2.4 Acetone

Fisher Optima.

6.3 Stock Standard Solutions

6.3.1 Mullins mix: 183 µg/mL

Aroclors
1232 = 75 µg/mL
1248 = 54 µg/mL
1262 = 54 µg/mL

6.3.2 Ultra mix:

Congeners
001 = 12 µg/mL
006 = 14.2 µg/mL
029 = 6.3 µg/mL
049 = 5.86 µg/mL

101 = 4.93 µg/mL
141 = 2.19 µg/mL
180 = 2.21 µg/mL
194 = 1.69 µg/mL
206 = 2.05 µg/mL
209 = 1.36 µg/mL

Additional Congener 001 will be added to the ultra mix due to it's low ECD response. Congener 001 was supplied by Ultra Scientific.

6.3.3 Trans-nonachlor

Accustandard solid.

6.3.4 Stable isotopes

Liquid, as is from Cambridge Isotopes.

6.3.5 Initial calibration standards

Dilutions of the Mullins mix listed above. Concentrations are listed under Section 6.8.

6.4 Sodium Sulfate

Anhydrous, granular - Mallinckrodt. Ash at 450°C for a minimum of four hours.

6.5 Alumina

60-325 mesh - Fisher Scientific. Ash at 450°C for a minimum of four hours. Store in oven at 105°C. When ready to use cool to room temperature. Weigh out needed mass into a round bottom flask. Add water to provide the necessary deactivation on a mass to mass basis. Stopper the flask with the appropriate sized ground glass stopper. Shake for two minutes. Wrap stopper and top of flask with parafilm and set in desiccator for 24 hours.

6.6 Silica Gel

60-200 mesh - Baker Analyzed. Ash at 300°C for a minimum of four hours. Store in oven at 105°C. When ready to use cool to room temperature. No deactivation is required for the procedure used in this method.

6.7 Calibration Standards

Standards are prepared at five concentrations by dilution of the stock standard with hexane. Concentrations correspond to the expected range of concentrations found in real samples and are in the linear range of the detector. Concentrations for the initial calibration standards are:
366 ng/mL, 586 ng/mL, 732 ng/mL, 1464 ng/mL, and 2928 ng/mL. The continuing calibration standard is 732 ng/mL.

6.8 Internal Standards

6.8.1 Source

Solids purchased from Ultra Scientific.

6.8.2 Concentration

#030 = 82.73 ng/mL
#204 = 59.16 ng/mL

6.8.3 Preparation

Individual stock solution of Congeners 30 and 204 were prepared in concentrations of 16,545 ng/mL and 11,831 ng/mL respectively in hexane. To a 100 mL volumetric flask 0.5 mL of each solution was dispensed via a 0.5 mL volumetric pipet. Hexane was added to the flask to bring to volume.

6.8.4 Procedure for addition to extracts

The 4 mL vial of internal standard solution will be removed from the freezer and allowed to come to room temperature. A drummond pipet of appropriate size will be cleaned and used in the transfer of internal standard to extracts. All extracts will be vortexed to ensure proper mixing.

6.8.5 Storage

Internal standard solutions are stored in amber glass bottles in a -16 ±4°C freezer.

6.9 Surrogate Standards

6.9.1 Source

PCB congener solids purchased from Ultra Scientific; stable isotope solutions purchased from Cambridge Isotopes.

6.9.2 Concentration

#014 = 439.2 ng/mL
#065 = 106.4 ng/mL
#166 = 125.04 ng/mL
^{13}C chlordane = 1317 ng/mL
^{37}Cl nonachlor = 1300 ng/mL

6.9.3 Preparation

Individual stock solution of Congeners 14, 65, and 166 were prepared in concentrations of 5.490 µg/mL, 5.320 µg/mL and 5.210 µg/mL respectively in hexane. To a 50 mL volumetric flask 4.0 mL of #14, 1.0 mL of #65 and 1.2 mL of #166 was dispensed via volumetric pipets. Hexane was added to the flask to bring to volume. The stable isotope solutions were diluted in hexane to appropriate concentrations in hexane.

6.9.4 Procedure for addition to samples

The 4 mL vial of surrogate solution will be removed from the freezer and allowed to come to room temperature. A drummond pipet of appropriate size will be cleaned and used in the transfer of surrogate solution to samples prior to extraction.

6.9.5 Storage

Surrogate standard solutions are stored in amber glass bottles in a -16 ±4°C freezer.

6.10 Matrix Spike Standard

6.10.1 Source

Mullins Mix.

6.10.2 Concentration

2928 ng/mL stock solution.

6.10.3 Preparation

The 2928 ng/mL Stock Spiking Solution will be diluted with hexane in the preparation of matrix spikes with concentrations that are within a factor of five of the media of interest. An appropriate amount of nonachlor will be added during this dilution step to be within a factor of five of the media of interest.

6.10.4 Procedure for addition to samples

The 4 mL vial of matrix spike solution will be removed from the freezer and allowed to come to room temperature. A drummond pipet of appropriate size will be cleaned and used in the transfer of matrix spike solution to the matrix prior to extraction.

6.10.5 Storage

Matrix spike standard solutions are stored in amber glass bottles in a -16 ±4°C freezer.

7.0 Sample Collection, Preservation and Handling

7.1 Collection

Details of sample collection are found in the SOP "Sampling Procedures for the Lake Michigan Lower Pelagic Foodchain for PCBs, Nonachlor and Mercury", Revision 1, 31 August, 1994.

7.2 Zooplankton

7.2.1 Definition

The zooplankton fraction is operationally defined as particulate matter greater than 100 µm (excluding fish). Approximately 10 g (wet weight) is needed for the analyses.

7.2.2 Collection

Wet zooplankton is removed from the net container and transferred to a clean glass bottle. The slurry in the glass bottle is poured through a piece of 100 µm Nitex netting supported by either a funnel or seive. The material remaining on the netting is removed by a spatula to an ashed glass jar.

7.2.3 Storage

Jars containing zooplankton are labeled and frozen until extraction.

7.3 Phytoplankton

7.3.1 Definition

The phytoplankton fraction is operationally defined as particulate matter between 10 and 100 µm. Approximately 10 g (wet weight) is needed for the analyses.

7.3.2 Collection

Wet phytoplankton will be quantitatively transferred from the net container to a clean glass graduated bottle. After a small subsample is provided for Hg analysis the remaining slurry is taken to the next nearest hundred mL with filtered lake water (i.e. slurry volume reads between 600 mL and 700 mL the volume is increased to 700 mL.).

7.3.3 Subsamples

The suspension is homogenized and subsampled for mass, taxonomic identification, organic carbon and stable isotopes. The remainder of the suspension is quantitatively transferred using filtered lake water and filtered through an ashed 125 mm GF/F glass fiber filter in a Buchner funnel by a gentle vacuum.

7.3.4 Storage

The glass fiber filter containing the phytoplankton will be folded in quarters, wrapped in foil, sealed in a freezer bag, labeled and frozen until extraction.

7.4 Detrital Fraction

7.4.1 Definition

The detrital fraction is operationally defined as the material collected between 0.7 and 10 µm, as isolated by a 293 mm GF/F glass fiber filter after passing through a piece of 10 µm Nitex netting. Approximately 1000 L of water must be processed for this size fraction.

7.4.2 Storage

The GF/F filters containing the detrital sample will be individually stored. Each filter will be folded in quarters, wrapped in aluminum foil, sealed in a freezer bag, and labeled and frozen until extraction.

7.5 Mysis Relicta

7.5.1 Samples of *Mysis relicta* are hand picked to ensure clean collections. Approximately 10 g (wet weight) is needed for the analyses.

7.5.2 Collection

Mysis are collected by vertical tows with 500 µm nets or by benthic sled tows. Material from these methods of collection are transferred to a pan for hand-picking of organisms. Organisms are placed in ashed glass jars.

7.5.3 Storage

Jars containing *Mysis* are labeled and frozen until extraction.

7.6 Diporeia sp.

7.6.1 Samples of *Diporeia sp*. are hand picked to ensure clean collections. Approximately 10 g (wet weight) is needed for the analyses.

7.6.2 Collection

Diporeia sp. are collected by benthic sled tows. Material from this method of collection are transferred to a pan for hand-picking of organisms. Organisms are placed in ashed glass jars.

7.6.3 Storage

Jars containing *Diporeia sp*. are labeled and frozen until extraction.

7.7 Holding Times

Samples are to be extracted within one year of collection, beginning after approval of the QAPjP. Extracts are stored in the freezer at -16 ±4°C in the dark and analyzed within three years after extraction.

8.0 Sample Preparation Procedure

8.1 Sample Selection

An appropriate sample is removed from the freezer and allowed to thaw. For biota samples aliquots are taken for weight wet and dry weight determinations.

8.2 Wet Weight Determination

An ashed glass beaker is placed on the balance and tared. An appropriate amount of sample is weighed into the beaker (between 5 and 10 g). The wet weight of the sample is recorded in the Lake Michigan Pelagic Foodchain notebook.

8.3 Dry Weight Determination

8.3.1 A small aliquot is taken from the remaining sample and placed in a tared aluminum foil cup. The wet weight is recorded. These aliquots are placed in a drying oven until they reach a consistent weight which is recorded in the notebook.

8.3.2 Phytoplankton dry weights are determined from SPM subsamples collected in the field. Nuclepore filters (0.4 µm) are allowed to air dry and their final consistent weight is recorded in the SPM field notebooks.

8.4 Methanol Wash

8.4.1 Set up a 1000 mL separatory funnel with a conical funnel on top. The conical funnel will hold a plug of ashed glass wool.

8.4.2 Transfer the whole wet sample from the beaker onto the glass wool with methanol (MeOH). Wash the sample with approximately 5-20 mL MeOH.

8.5 Methanol Extraction

8.5.1 Transfer both the sample and glass wool from the conical funnel to a Soxhlet extractor apparatus containing a plug of ashed glass wool. Sample is transferred with methanol.

8.5.2 In the case of filters, the filters will be carefully cut into pieces and the pieces will be added to the Soxhlet extractor. Cutting will be done over the aluminum foil the filters were wrapped in and the aluminum foil will be rinsed with methanol with the methanol rinse added to the Soxhlet.

8.5.3 Add approximately 1 tablespoon ashed boiling stones to a 500 mL round bottom flask.

8.5.4 Connect the round bottom to the Soxhlet extractor.

8.5.5 Add approximately 300 mL MeOH through the Soxhlet so it completely covers the sample and drains to the round bottom flask.

8.5.6 Add the appropriate amount of surrogate standard solution. This is dependent on the matrix being extracted - it will vary from 50-200 L. The lab procedural blank will be spiked with an analogous amount of surrogate.

8.5.7 Connect the Soxhlet extractor to the condenser.

8.5.8 Turn on the heating mantel. Let cycle for four hours.

8.5.9 After four hours turn the heating mantel off and let sample cool until round bottom flasks are warm.

8.5.10 Quantitatively transfer the MeOH extract from the round bottom flask to the separatory funnel set up in Section 8.4.

8.6 Dichloromethane Extraction (DCM)

8.6.1 Recharge the round bottom flask with approximately 300 mL DCM.

8.6.2 Reattach the round bottom to the Soxhlet extractor.

8.6.3 Cycle for 16-24 hours.

8.6.4. Allow the extract to cool to room temperature after the extraction is complete.

8.7 Batch Extraction of Methanol Fraction

8.7.1 To the separatory funnel containing the MeOH fractions add the following:

100 mL Barnstead Nanopure water
50 mL saturated NaCl solution
50 mL hexane

8.7.2 Shake separatory funnel for three minutes. Vent funnel often.

8.7.3 Drain lower water layer to a large bottle.

8.7.4 Drain hexane layer into a KD body connected to a receiver holding a funnel containing approximately 150 g sodium sulfate (Na_2SO_4) on top of a plug of ashed glass wool. Wash the Na_2SO_4 with approximately 2 x 15 mL hexane.

8.7.5 Pour the water from Section 8.7.3 back into its corresponding separatory funnel.

8.7.6 Rinse the bottle with 50 mL hexane and add to separatory funnel.

8.7.7 Shake separatory funnel for three minutes, and drain lower layer as above. Add the hexane layer to the KD from Section 8.7.4. Repeat one more time starting at Section 8.7.5. Wash separatory funnel with 3 x 15 mL hexane on the last extraction.

8.8 Volume Reduction and Solvent Exchange

8.8.1 Attach a three-ball Snyder column to the KD assembly. Place on steam bath and adjust the vertical position of the assembly so that the appropriate flux is accomplished. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

8.8.2 Reduce the MeOH fraction to approximately 10-15 mL.

8.8.3 Quantitatively transfer the DCM extract obtained from Section 8.6 to the KD apparatus.

8.8.4 Volume reduce to approximately 15 mL.

8.8.5 Solvent exchange the extract to hexane by adding 30 mL hexane, reducing volume to 15 mL, adding another 30 mL hexane, reducing volume to 15 mL and adding a final 15 mL hexane.

8.8.6 Reduce the volume to 10-15 mL. Turn off the steam tables, cool the KD assembly to room temperature and stopper with a ground glass stopper of appropriate size.

8.8.7 Remove the KD body from the receiver - rinse the ground glass joint with hexane into the receiver.

8.8.8 Volume reduce the extract to less than 10 mL using a Nitrogen evaporation apparatus. Needles are sonicated in hexane prior to use.

8.8.9 Transfer, with hexane, the extract to a 25 mL graduated cylinder.

8.9 Lipid Content Determination

8.9.1 Add hexane to the graduated cylinder to bring extract to 10 mL. Using a 1 mL volumetric pipet, transfer 1 mL of the extract to a pre-weighed aluminum tin.

8.9.2 Evaporate solvent from the aluminum tin by air exposure.

8.9.3 After solvent has evaporated, weigh the aluminum tin to constant weight using the Sartorius MC1 balance. Residue remaining in the tin is the lipid.

8.10 Lipid Removal

8.10.1 Column is assembled in the following fashion from top to bottom.

2 g ashed sodium sulfate
13 g 6% deactivated alumina
1 g ashed sodium sulfate
Plug of ashed glass wool
Wash columns with 2 x 60 mL hexane

8.10.2 Deactivation

See Section 6.6 for alumina deactivation procedure.

8.10.3 Columns

1 x 50 cm columns.

8.10.4 Quantitatively load the extract to the top of the column using hexane.

8.10.5 Elute with 3 x 50 mL hexane and collect in a 250 mL KD body attached to a receiver containing two to three ashed boiling chips.

8.10.6 Attach three ball Snyder columns to the KD body and place the assembly on the steam table. Adjust vertical height for appropriate flux.

8.10.7 Reduce the extracts to approximately 5-10 mL using the same procedure described in Sections 8.8.4 through 8.8.7.

8.11 Column Cleanup/Fractionation

8.11.1 Cleanup/fractionation column is assembled in the following fashion from top to bottom.

3 g ashed sodium sulfate
4.5 g 0% deactivated silica
1 g ashed sodium sulfate
6 g 1% deactivated alumina
1 g ashed sodium sulfate
Plug of ashed glass wool
Wash columns with 2 x 50 mL 2% DCM/hexane, 2 x 50 mL 40% DCM/Hexane, 2 x 60 mL 100% hexane

The silica is made into a slurry using hexane before it is poured into the column. Pouring silica in this fashion results in columns that pack better and have fewer air channels.

8.11.2 Deactivation

See Section 6.6 for alumina deactivation procedure.

8.11.3 Columns

1 x 50 cm columns.

8.11.4 Quantitatively load the extract to the top of the column using hexane.

8.11.5 Elute with 95 mL 100% hexane and collect in a 250 mL KD body attached to a receiver containing two to three ashed boiling chips. This is labeled the F1 fraction containing PCBs.

8.11.6 Elute again with 105 mL 40% DCM in hexane and collect in a different 250 mL KD body attached to a receiver containing two to three ashed boiling chips. This is labeled the F2 fraction containing trans-nonachlor and toxaphene.

8.12 Final Solvent Exchange

8.12.1 Attach three ball Snyder columns to the KD body and place the assembly on the steam table. Adjust vertical height for appropriate flux.

8.12.2 Reduce the extracts to approximately 5-10 mL using the same procedure described in Sections 8.8.4 through 8.8.7.

8.12.3 Reduce to a final volume of 2-3 mL using the procedure described in Section 8.8.8.

8.12.4 Quantitatively transfer the extract using hexane from the receiver to ashed 4 mL amber vials.

8.13 Storage

Store extract in the freezer at -16 ±4°C until internal standards addition and analysis.

8.14 Internal Standards Addition

8.14.1 Remove extracts from the freezer.

8.14.2 Remove internal standards solution from freezer and allow to come to room temperature.

8.14.3 Reduce extract to approximately 300 µL using nitrogen evaporation apparatus.

8.14.4 Add 50-200 µL internal standard solution (#30, #204) depending on the matrix to the PCB extract fraction using a Drummond pipet and 50-200 µL of chlordane (matrix dependent) to the trans-nonachlor extract fraction via Drummond pipet.

8.14.5 Vortex the extracts. The extract are now ready for instrumental analysis.

9.0 Instrument Calibration and Quantitation

9.1 GC/ECD Analysis for PCB Congeners

9.1.1 Annual Initial Calibration

The GC/ECD will be calibrated at the beginning of the project (or after any major instrument repairs such as replacements of column, detector, or injector assembly) by using calibration standards prepared at five different concentrations as listed in Section 6.8. The concentrations correspond to the expected range of concentrations in the samples or define the working range of the detector. Each calibration standard is injected using the technique that will be used for the injection of environmental extracts. Peak areas are tabulated against the mass injected for each congener and associated internal standard area and mass.

Note: Because of the sensitivity of the electron capture detector, the injection port and column should always be cleaned prior to performing the initial calibration.

9.1.1.1 Relative Response Factor (RRF)

Millennium Chromatographic Management System automatically calculates the RRF each time it calibrates a calibration standard. The calculation used for this is as follows:

$$RRF = (C_s/A_s) \;/\; (C_{is}/A_{is})$$

Where A_s = *area of the congener*
A_{is} = *area of the internal standard*
C_{is} = *mass (ng) of the internal standard*
C_s = *mass (ng) of the congener*

9.1.1.2 Relative Standard Deviation (RSD)

The math for this calculation across all five RRFs is as follows:

$$RSD = sd \;/\; X_{RRF} \;x\; 100$$

Where *sd = standard deviation of the five RRF measurements*
X_{RRF} = *mean RRF across all five RRFs.*

9.1.1.3 If RSD value for more than 5% of the congeners exceeds 25%, the GC system will be reoptimized and calibration will be performed under the new conditions.

9.1.2 For calibration verification a midrange concentration standard containing all congeners, surrogates and internal standards will be injected as a Continuing Calibration Standard with each set of samples run on the GC.

9.1.2.1 Each congener RRF calculated by Millennium is compared to the mean RRF from the initial calibration. A relative percent difference (RPD) of the continuing calibration RRF from the initial calibration mean RRF for each congener is determined as follows:

$$RPD = (RRF_{cc} - X_{RRF\ ic} / X_{RRF\ ic}) * 100$$

Where RRF_{cc} *= RRF for continuing calibration standard*
$X_{RRF\ ic}$ *= mean RRF from the initial calibration*

9.1.2.2 If the % difference exceeds 100% for more than 30% of the congeners, the GC system will be inspected to determine the cause. Any required maintenance will be performed and calibration will be reverified. If routine maintenance does not correct the GC performance based on the annual initial calibration, a new initial calibration will be performed.

9.2 Retention Time Windows

9.2.1 Retention time (RT) windows can be established only when the GC is operating under optimum conditions. Any drift in retention times result in meaningless RT windows. The width of RT windows is established on a run by run basis. Each calibration standard run at the beginning of a sample set is used to establish RT windows. These RT windows are confirmed on the performance standard run at the end of the sample set.

9.2.2 If the RT windows determined from the calibration standard fail to identify peaks correctly in the performance standard, it will be necessary to perform maintenance steps.

9.2.3 Check for leaks throughout the GC system. This problem can cause large and continuing shifts of all peak retention times. Check for a column blockage leading to skewed and/or deformed peaks.

9.2.4 Once maintenance has been performed the sample set will be rerun. If the problem is still not corrected it may be necessary to change the column.

9.2.5 Retention time windows are recalculated when columns are clipped or new columns are installed.

9.3 GC Analysis of Samples

9.3.1 Samples are analyzed in a set referred to as a sample set. The sequence will always begin with a hexane injection followed by a continuing calibration check, sample extracts, field blanks, procedural blank and a performance standard. A typical run sequence would look like this:

Vial	Description
1	Hexane
2	Standard
3	Sample
4	Sample
5	Sample
6	Sample

7	Sample
8	Proced. Blank
9	Performance Standard

9.3.2 Continuation of sample injection may continue for as long as the daily continuing calibration standard and Mullin mix standard interspersed with the samples meet QC requirements. It is recommended that standards be analyzed at least after every 20 environmental samples and at the end of a set.

9.3.3 Each sample set will be bracketed with an acceptable continuing calibration standard and a performance standard.

9.3.4 Baselines for the continuing calibration standard are carefully drawn, peaks are identified and daily absolute RT windows for each congener are established. Congener identification occurs when a peak from a sample extract falls within the daily retention time window.

9.3.5 Millennium automatically performs calculation of peak mass using the internal standard calibration procedure as follows:

$$PCB_{mass} = Area_{PCB} \times RRF \times [(mass_{istd})/(area_{istd})]_{sample}$$

Where PCB_{mass} = *pg PCB congener detected in sample*
$Area_{PCB}$ = *area of PCB congener detected in sample*
RRF = RRF of PCB congener from continuing calibration std
$mass_{istd}$ = *pg applicable internal standard added to sample*
$area_{istd}$ = *area of internal standard response in sample*

9.3.6 If the peak response is less than the SDL the validity of the quantitative result may be questionable. The sample will be analyzed to determine if further concentration is warranted.

9.4 GC/MS/ECNI Analysis of Trans-nonachlor

9.4.1 Tuning

The GC-MS is tuned approximately every two to three weeks. The decision to re-tune the instrument is based on evaluating a daily injection of the performance standard octafluoronaphthalene (OFN). Peak area, shape, and electron multiplier setting (sensitivity) are evaluated by a trained operator. If re-tuning is necessary the instrument is re-tuned in negative chemical ionization mode.

9.4.2 Initial calibration

Consists of three solutions of perfluorotributylamine (PFTBA) at concentrations ranging from 10 ng/mL to 75 ng/mL. Criteria include m/z 633 = 50,000 and m/z 452 should equal

3-15% of m/z 633. The calibration is done at the beginning of the project, and anytime after source vacuum has been lost and re-gained due to cleaning or repairs.

9.4.3 Continuing Calibration

A trans-nonachlor standard will be run with each sample set.

9.4.4 RT Window and SIM criteria - retention time window is set based on the trans-nonachlor standard run as the continuing calibration standard. The standard is run under full scan and the retention time for trans-nonachlor is determined. Within this retention time the program monitors for ions corresponding to the mass of trans-nonachlor - 444 m/z and 442 m/z. The m/z 444 is the quantitation ion, m/z 442 is the confirmation ion. Samples will be checked within the retention time window for the appropriate ions.

9.4.5 Analysis sequence

Samples are analyzed in sample sets. A typical run sequence appears as follows:

Vial	Description
1	Hexane
2	Standard
3-8	Samples
9	Standard
10-15	Samples
16	Standard

9.4.6 Baselines

Baselines are manually set using the HP Aquarius software program.

9.4.7 Quantitation

Areas are determined by Aquarius, and the mass of trans-nonachlor is calculated using the internal standard method as described in Section 9.3.5.

10.0 Preventative Maintenance

10.1 GC

10.1.1 Columns

When installing a column, it is very important to scrutinize the cut very carefully as jagged or angled cuts can result in poor chromatography or gas leaks.

10.1.2 Gases

Gases go through a series of filters before reaching the GC including; moisture trap, hydrocarbon trap, oxygen trap, indicating oxygen trap and chemical filter. Only Ultra-Pure Carrier grade hydrogen and helium with purity of 99.999% should be used.

10.1.3 Septa

Septa should be changed with each run. Only teflon septa should be used with the GC/ECD.

10.1.4 Injector

If chromatography indicates an injection side problem it will be necessary to clean the injector port and replace the injector liner. At this time it is also a good idea to clip the column and reinstall.

10.1.5 ECD

Since the ECD contains a radioactive compound (^{63}Ni) it is regulated by the Nuclear Regulatory Commission. All maintenance relating to the ECD is handled by the University of Minnesota Radiation Protection Division.

10.2 MS

10.2.1 Mechanical pump oil is changed bi-annually.

10.2.2 Refrigerant coolant level is checked once every three months.

10.2.3 The source is cleaned as needed determined by chromatography quality.

10.2.4 Poles and ceramics are cleaned as needed.

10.2.5 Fan screens are cleaned and the area surrounding the instrument is vacuumed on a monthly basis.

10.2.6 Cleaning contacts on the component boards and vacuuming component boards is covered though an HP service contract for the instrument.

10.3 Balances

10.3.1 Sartorius MC1 balance has internal calibration ability. Before weighing, an internal calibration is performed. Weights ranging from 2 mg - 1 g are used to double check the calibration.

10.3.2 Balances are checked before weighing for cleanliness. Solids wedged under the weighing pan can result in unstable readings.

11.0 Quality Control Requirements

11.1 Surrogate Recovery

11.1.1 Surrogate standard recovery in all samples, blanks, and spikes will be calculated. Samples with average surrogate spike recoveries <50% or >125% will be re-analyzed or flagged. The following steps will also be investigated.

11.1.2 Confirm that there are no errors in amounts of surrogate solutions or internal standards added to the solution.

11.1.3 Examine chromatograms for baseline determination and interfering peaks.

11.1.4 Recalculate the data if any of the above checks reveal a problem.

11.1.5 Reextract and reanalyze the sample if none of the above are a problem. If an individual surrogate standard exceeds the stated limits, the surrogate will be flagged as FSS. If two of three surrogates exceed the QA limits, all data for that sample will be flagged FSS.

11.2 Calibration QC

11.2.1 Initial Calibration

An initial calibration will be performed annually. Five concentrations of all congeners will be used and the RRF RSD across all five concentrations will be <25% for 95% of the congeners in order for the calibration to be considered valid. If this criteria is not met, recalibration must occur until it is. If problems persist, the stability of the standards used and the dilution techniques should be checked.

11.2.2 Continuing Calibration

A continuing calibration standard will be analyzed and compared against the initial calibration with each run. The RRF RSD of each congener from the initial calibration RRF must be <100% in order for sample analyses to occur. If this criteria is not met, action must be taken to achieve an acceptable calibration prior to resuming sample analysis.

11.3 Internal Standards Performance

Internal standard RF and 204/30 ratio in each sample, blank and standard will be evaluated for acceptance. The change in each internal standard's RF in each sample compared to the continuing calibration RF must be <50%. The change in ratio of 204/30 area response for each sample compared to the continuing calibration ratio must be < 50%. If the ratio fails this criteria, then the absolute RF of each internal standard is examined to determine of one or both internals standards are compromised (change in RF >50%). If one internal standard in the PCB analysis is compromised, the other internal standard should be used for quantitation. If both fail the sample will be rerun or all sample data will be flagged FIS. If #204 for GC/MS/ECNI analysis of trans-nonachlor fails the criteria of the change in RF compared to the continuing calibration (RF <50%) the sample will be rerun or all data will be flagged FIS.

11.4 Blanks

11.4.1 Lab Procedure Blank

A lab procedure blank will be prepared with every six samples. This blank consists of all reagents, surrogates and internal standards used in the extraction of environmental samples at the volumes used in these extractions. The procedure blank is carried through the entire analytical procedure in the same manner as a sample. Any detected congeners at a concentration <SDL will be flagged FBK. If the blank exceeds this criteria for more than

30% of the congeners in the samples, the samples may be flagged following evaluation of the data by the analyst. Also, all reagents will be checked before proceeding with additional analyses and the associated sample sets will be checked against previous ones for self consistency. If the sample data or reagent purity are questionable, samples will be re-extracted or flagged FKB if no further sample is available. If sample data are consistent with previous data and reagent blanks are acceptable then the data will be accepted without flagging.

11.4.2 Field Blanks

Collection of field blanks will occur at a frequency of two blanks per 11 phytoplankton/detrital/XAD fraction samples collected. Any detected congeners at a concentration <SDL will be flagged FBK. If the blank exceeds this criteria for more than 30% of the congeners in the samples, the samples may be flagged following evaluation of the data by the analyst, as above.

11.5 Duplicates

11.5.1 Lab Duplicates

Zooplankton samples will have a lab duplicate prepared at a frequency of one per 11 samples/cruise. One zooplankton sample from each cruise will be split in the lab and analyzed as a duplicate. The Relative Percent Difference (RPD) between the sample and its lab duplicate must be <30% for 70% of all congeners. If this RPD is exceeded, associated sample data will be flagged as FDL.

11.5.2 Field Duplicates

Collection of field duplicates will occur at a frequency of two per 11 samples/cruise except for *Diporeia* and *Mysis* which have a frequency of one per 11 samples/cruise. The RPD between the sample and its field duplicate must be <30% for 70% of all congeners. If this RPD is exceeded, associated sample data will be flagged as FFD.

11.6 Matrix Spikes

Mullins Mix solution will be added to result in a concentration within a factor of five of the media of interest to one sample out of every 24 extracted and analyzed. The percent recovery of 70% of all congeners will be between 50-125%. If recovery is outside of these limits, the associated sample data will be flagged as FMS.

11.7 Performance Standard

Mullins mix (1994) at a concentration comparable to the quantitation standard will be analyzed with every sample set. The recoveries of the congeners must be between 90-110% for 70% of all congeners for the associated sample data to be considered valid. If recovery is outside of these limits, associated sample data for that congener should be flagged as FPC.

11.8 Retention Time Window

Analytes reported as detected must be within the retention time window as described in Section 9.2. If the analyst believes that a retention time window shift has occurred and the analytes are present outside the statistically established window, maintenance on the instrument will be performed and the sample set rerun.

12.0 Data Reporting

12.1 Units

All PCB congeners and trans-nonachlor will be reported as pg/g dry weight calculated as:

$$pg/g\ PCB\ or\ trans{-}nonachlor = [A_s\ x\ RRF\ x\ M_{is}]\ /\ [A_{is}\ x\ W_s]$$

Where A_s *= area counts of the analyte in the sample*
RRF = relative response factor of the analyte based on the continuing calibration standard
M_{is} *= pg of internal standard added to the sample*
A_{is} *= area counts of internal standard in the sample*
W_s *= weight of sample extracted, in g dry weight*

12.2 PCBs

12.2.1 Total PCBs as a sum of all congeners, the sum of each homologe series and each congener concentration will be reported.

12.2.2 A surrogate correction value will be reported in the appropriate field of the "Laboratory Reporting Standard". Raw data will be divided by this value to provide the reported result value.

Surrogate Recovery Correction: Each congener concentration will be corrected to the recovery of the surrogate that best represents that congener. This is determined by running duplicate procedural spikes, and correcting the recoveries to each of the three surrogate standards. The region of the chromatogram that is corrected closest to 100% by a given surrogate would be the most appropriate surrogate for those congeners. In past projects, the first third of the chromatogram was best corrected to Congener 14, the middle third to Congener 65, and the final third to Congener 166 (see Table 6, QAPjP). This study will be conducted specifically for this project.

12.3 Data

Data from Millennium and GC/MS/ECNI data system Aquarius will be electronically transferred to spreadsheets in Excel for QA review and data reduction/calculation.

12.4 Associated QC

Results of lab/field blanks, lab/field duplicates, matrix spike recoveries, surrogate spike recoveries, and performance standard recoveries will be reported with each sample batch.

13.0 Method Validation Procedures

13.1 Analyst Proficiency

Analysts associated with this project have reviewed the Sampling SOP, the Sample and Analysis Quality Assurance Project Plan and this Analysis SOP. Analysts working with either the GC or the GC/MS/ENCI have extensive experience in operating, maintenance, and repair of the instrument. Additionally, analysts have a strong working knowledge of the software used in analyzing the data associated with each instrument.

13.2 Detection Limit Determinations

13.2.1 IDL

The annual initial five-point calibration curve will be constructed and extrapolated to determine the y axis intercept. This intercept will be considered the Instrument Detection Limit (IDL).

13.2.2 MDL

The Method Detection Limit (MDL) for each homologue series will be determined once during the project using the Ultra standard mix at a low level concentration in a procedural spike. A mean value and standard deviation for each congener in the Ultra mix will be established across seven spikes. The Ultra MDLS are equal to three times the standard deviation. The Ultra MDL for a given homolog will be proportioned against each congener's RRF within a homolog to establish a congener specific MDL. The congener specific RRF will be determined from PCB calibration standards run on the GC the same day as the MDL spiked samples. These MDLs will be used in data reporting.

13.2.3 SDL

The System Detection Limit (SDL) will be calculated annually. This will occur once seven field blanks have been analyzed. The SDL will be set as +3sd of the mean field blank concentration.

14.0 References

14.1 Mullin, M. 1985. PCB Congener Workshop, Large Lakes Research Station, U.S. EPA, Grosse Ile, Michigan.

14.2 Pearson, R.F., K.C. Hornbuckle, K.A. Golden, S.J. Eisenreich, and D.L. Swackhamer. 1996. PCBs in Lake Michigan Water: Comparison to 1980 and mass budget for 1991. *Environ. Sci. Technol*. 30:1429-1436.